follow, it is respectfully submitted that the instant application is in condition for allowance and such action is earnestly solicited.

The §103 Rejection

The Examiner has rejected claims 1-22 under 35 U.S.C. §103 as being obvious over de Boer, et al., U.S. patent no. 5,518,751 ("de Boer"), in view of Cook, et al., U.S. patent no. 5,554,646 ("Cook"). It is the Examiner's position that de Boer teaches that CLA in food compositions such as milk are useful in treating disorders such as diabetes. The Examiner points to column 1, lines 35-43 in support of that view. The Examiner recognizes that de Boer does not teach particularly that CLA is useful in a method of treating diabetes, the specific conjugated linoleic acids claimed or the amount of CLA of the present invention. The Examiner cites Cook for teaching a method of adding linoleic acid compounds into animal feed to reduce fat in an animal and that specific isomers of octadecadienoic acid may be included in the conjugated linoleic acid.

From the teachings of the cited art the Examiner concludes that it would have been obvious to employ CLA in a method of treating diabetes and that it would have been obvious for one of ordinary skill in the art at the time the invention was made to incorporate about 1 mg to about 10,000mg/kg of body weight of the *trans,cis*-9,11-octadecadienoic acid, *cis,cis*-9,11-octadiendioic acid or *trans,cis*-10,12-octadecadienoic acid into a milk composition product useful in a method of treating diabetes. The Examiner further argues that one of ordinary skill would have been motivated to employ CLA in a method of treating diabetes because de Boer, et al. clearly teaches unsaturated fatty acids, including CLA, are useful in treating disorders such as diabetes. It is the Examiner's conclusion, therefore, that one of ordinary skill would have reasonably *expected* that CLA would have been useful in a method of treating diabetes. Applicants respectfully traverse the Examiner's rejection.

It is respectfully submitted that the Examiner has not made out a cogent case for obviousness. Indeed, it is respectfully submitted that the Examiner has seized on a brief, *ambiguous* statement in the background section of a U.S. patent to de Boer which does not teach or suggest the use of CLA for treating diabetes and combined that ambiguous disclosure with the teachings of Cook which are directed to using CLA to prevent or treat the adverse effects of antibody based hypersensitivity (attenuating the allergic response of Animals), to argue that the claimed invention is obvious. As will be argued in detail herein, the Examiner's argument is not cogent and represents a rejection based upon an impermissible *hindsight* reconstruction of the teachings of the two references in rendering the present invention obvious. A combination of the teachings of the references relied upon by the Examiner, without reliance on a *hindsight* reconstruction, which is invalid under the law, would not result in a finding that the present invention was unpatentable. See, Michael L. McGinley v. Franklin Sports, Inc., 262 F.3d 1339, 60 USPQ2d 1001 (Fed. Cir. 2001), a copy of which was previously submitted to the Examiner.

de Boer Does Not Teach or Suggest the Use of CLA for Treating Diabetes

Contrary to the Examiner's conclusions regarding the teachings of de Boer, de Boer ***does not*** disclose or suggest the use of CLA for the treatment of diabetes. In contrast, de Boer merely reiterates and summarizes the state of the art at the time of the filing of de Boer (September 8, 1994), ***which did not recognize the significance of conjugated linoleic acid in the treatment of diabetes.*** Even the Examiner admits this in the office action on page 3, first paragraph. Contrary to what the Examiner has posited in making the rejection, the art clearly did not teach or suggest the use of CLA as a treatment modality for diabetes. Indeed, prior to the present application, it was not known that CLA, in contrast to γ-linolenic acid (GLA, commonly found in evening primrose oil, for example), could be used to treat diabetes. Based upon the state of the prior art at the time of the filing of deBoer on September 8, 1994, the passage in de Boer relied upon by the Examiner (namely, the fourth paragraph of column 1 of de Boer) can be taken to mean that fatty acids disclosed in that paragraph *which were taught in the prior art to be useful for treating*

diabetes could be used to treat diabetes. At that time, however, and as explained in great detail in the previous communications with the Patent Office, those fatty acids did not include CLA as presently claimed, but did include some of the other fatty acids which were cited in the ambiguous passage of de Boer. Based upon the foregoing, it is respectfully submitted that the passage in de Boer relied upon by the Examiner represents a recitation of the prior art at the time of the filing of the de Boer application, which art taught that certain fatty acids disclosed by de Boer in the ambiguous passage could be used to treat diabetes, but *failed to recognize that CLA was a particularly effective treatment of diabetes.* It is further noted here that de Boer is otherwise inapposite to the present invention inasmuch as it relates to methods for the preparation of milk and milk powders having a long storage life by adding a fat fraction containing unsaturated fatty acids to the milk liquid.

The Examiner has cited no art, separate from the ambiguous disclosure in the background section of deBoer, which even arguably teaches or suggests the use of CLA for the treatment of diabetes. Thus, in context, given the ambiguous nature of the disclosure on which the Examiner relies, the Examiner's construction of the ambiguous disclosure must fail. Moreover, it is respectfully submitted that the Examiner is clearly engaging in ***impermissible hindsight reconstruction*** of the ambiguous teachings of the art to somehow justify that the present invention is obvious. Note that at the time of the filing of the application, certain fatty acids were known to be useful in the treatment of diabetes (see above). However, prior to the present application, CLA was not known to be useful for treating diabetes, although it was known for the treatment of other conditions, which are actually set forth in the disclosure of deBoer. Indeed, the Examiner must contort the ambiguous disclosure of deBoer because the art actually failed to appreciate the present invention.

Prior to the present application, CLA was not known as a treatment modality for diabetes, and indeed, the first report in the literature of the significance of CLA in the treatment of diabetes, was Applicants' own paper, *Biochem Biophys Res Commun,* March 27, 244(3) 678-682

(1998). The date of Applicants' paper is some four (4) years after the filing date of de Boer. Prior to the present application, CLA was known for its anti-carcinogenic and anti-atherogenic properties having cardiovascular implications. Also known in the art was that α-linolenic acid and linoleic acid possesses properties which make it potentially useful in the treatment of cardiovascular disease (as indicated by de Boer). Thus, the disclosure in de Boer at column 1, lines 35-43, is completely consistent with the conventional understanding at the time of the filing of that reference and refers to the fact that it was known in the art to use linolenic acid in cardiovascular diseases and diabetes. It was, however, not known in the art before the present invention, that CLA could be used for the *treatment of diabetes or that CLA administration is a particularly effective treatment for diabetes.* Despite Applicants' requests in previous papers to have the Examiner cite *any reference* other than deBoer in support of the contention that the use of CLA in the treamtent of deBoer teaches the use of CLA, the Examiner has not been able to cite such a reference. That is because such a reference does not exist.

The ambiguous passage in de Boer, which the Examiner relies on for the teaching that CLA may be used to treat diabetes is found in the background of the invention section at column 1, lines 35-43 and is presented below:

"An important reason for enriching milk or milk powders with fats containing a high percentage of unsaturated fatty acids or strongly unsaturated fatty acids is to prevent or reduce cardiovascular diseases, atrophies, rheumatic disorders or diabetes. In particular, such products contain a high percentage of oleic acid, linoleic acid which may or may not be conjugated, α-linolenic acid and unsaturated C_{20} and C_{22} fatty acids."

A fair reading of that ambiguous passage in the BACKGROUND OF THE INVENTION section of de Boer is that de Boer is merely reviewing the conventional understanding at the time of the filing of de Boer which failed to appreciate the particularly effective use CLA could have in treating diabetes. Thus, it may be accurately argued and concluded, that de Boer merely reiterates a broad discussion of the art which did not teach or suggest the use of CLA for the

treatment of diabetes, but rather the use of one or more of the disclosed fatty acids to treat the indicated conditions. It is again noted that, despite Applicants' several requests, the Examiner has failed to clear up the ambiguous disclosure of deBoer and has not separately cited any reference which actually teaches or suggests the use of CLA for the treatment of diabetes and instead, relies on the deficient disclosure of de Boer to make the rejection. Thus, de Boer does not teach the use of CLA for the treatment of diabetes, because the use of CLA to treat diabetes was first disclosed in the present application, well after de Boer. Again, Applicants challenge the Examiner to clarify the ambiguous disclosure of de Boer and actually cite a prior art reference which teaches the use of CLA for the treatment of diabetes. Note that the main aspects of de Boer are otherwise irrelevant to the present invention inasmuch as de Boer relates to methods for the preparation of milk and milk powders having a long storage life by adding a fat fraction containing unsaturated fatty acids to the milk liquid. Diabetes is not otherwise even obliquely mentioned.

De Boar never proposed that CLA could lower glucose. Lowering glucose (fasting, post-prandial, post-absorptive--- is the main goal of the treatment of diabetes (see American Diabetes Association; www.diabetes.org). The bottom line is de Boar never speculated, taught or understood that CLA could improve glucose control, i.e, could actually treat diabetes. To the inventors' knowledge, there has never been a study to show that any fatty acid (CLA, n-3 PUFA, n-6-PUFA, etc) other than the current inventor's work (the present application as well as the inventors' papers published after the present application) with ***CLA, improves glucose control*** and thus represents a viable treatment for diabetes. This was not taught by or known at the time of de Boer.

Cook Does Not Obviate the Deficiencies of de Boer

Turning to the disclosure of Cook, this reference discloses a method of using CLA to reduce an immunogenic (allergic) response in animals, including humans. Although this

reference supports the view that CLA may be used to attenuate a humoral immunogenic response (and thus, allergies) and/or to increase the white blood cell count in a mammal, there is absolutely no disclosure or suggestion of the use of CLA in the treatment of diabetes. Indeed, other than the fact that Cook suggests that CLA may be included in an animal's diet, the relevance of Cook has not been made clear to Applicants.

It is respectfully submitted that a combination of de Boer and Cook does not disclose or suggest the present invention and that these references, in combination, only become relevant to the present invention *after one has read the instant application*. Thus, the Examiner's rejection is an example of impermissible hindsight reconstruction, a rejection which is clearly impermissible under the law. See MPEP §706.02(j) and *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). This is especially true where, as here, the prior art, *in general*, does not disclose or suggest the claimed invention, and the Examiner relies for such teaching, on an ambiguous description of the prior art. As was stated in *In re Vaeck*, "the teaching or suggestion to make the claimed combination and the reasonable expectation of success must be found in the prior art and not based on applicant's disclosure." *In re Vaeck*, at 20 USPQ2d 1438,1442.
In the present application, the Examiner has seized upon the ambiguous disclosure in de Boer, and **with impermissible reference to Applicants' disclosure and teachings**, has concluded that de Boer teaches the present invention. ***The Examiner's analysis therefore represents a classical case of hindsight reconstruction to make an obviousness rejection- an analysis which the Court of Appeals for the Federal Circuit has found to be impermissible***.

See also the CAFC's discussion of hindsight reconstruction in the previously enclosed McGinley case on page 12, in the first full paragraph.

> "The genius of invention is often a combination of known elements which in hindsight seems preordained. To prevent hindsight invalidation of patent claims, the law requires some 'teaching, suggestion or reason' to combine cited references. *Gambro Lundia AB v. Baxter Healthcare Corp.*, 110 F.3d 1573, 1579, 42 U.S.P.Q.2D 1378, 1383 (Fed. Cir. 1997). When the art in question is relatively simple... the opportunity to judge by

hindsight is particularly tempting. Consequently, the tests of whether to combine references need to be applied ***rigorously***. (Emphasis ours). See *In re Dembiczak*, 175 F.3d 994, 999, 50 U.S.P.Q.2D 1614, 1617 (Fed. Cir. 1999), limited on other grounds by *In re Gartside*, 203 F.3d 1305, 53 U.S.P.Q.2d 1769 (2000) ***(guarding against falling victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher)***." (Further emphasis ours).

In the present application, there is simply no cogent basis upon which to suggest that the prior art taught the use of CLA for the treatment of diabetes. While the disclosure in de Boer is unclear, the remaining art cited, Cook, is *inapposite* to the teaching. Yet, the Examiner, recognizing the deficiencies in the art, does not separately posit a prior art reference which teaches the use of CLA for the treatment of diabetes- that is because no independent basis exists. Applicants have respectfully requested the Examiner to cite whatever prior art may be available for the teaching that CLA is a particuarly effective treatment for diabetes, independent of the ambiguous disclosure of de Boer. If the Examiner cannot make such a recitation, Applicants respectfully request the Examiner to withdraw the rejection of the present application.

The Examiner has also maintained his rejection of claims 1-22 under 35 U.S.C. §103 as being obvious over Semenkovich and Heinecke, *Diabetes*, 1997, 46:327-334 ("Semenkovich"), in view of Steinhart, *Journal of Chemical Education*, 1996, 73(12):A302 and Cook (see above).

In sum, the Examiner cites Semenkovich for teaching that most diabetic patients die from macrovascular complications and that oxidative modification of lipoproteins in diabetic patients is enhanced, with this being one of the major risks for developing cardiovascular complications (macrovascular complications) in diabetic patients. Semenkovich is also cited for teaching that antioxidants are potent inhibitors of lipoprotein lipid peroxidation and thereby reduce the lipoprotein oxidation products and cytotoxicity caused by those products. The Examiner acknowledges that Semenkovich does not expressly teach the employment of CLA in a method to treat diabetes or the symptoms of diabetes or the specific isomers of octadecaienoic acid or

amounts of CLA.

The Examiner cites Steinhart for teaching CLA as a natural antioxidant. Cook is cited for teaching a method of adding CLA to animal feed.

From the disclosures of Semenkovich, Steinhard and Cook as set forth in the office action, the Examiner contends that the present invention is obvious and therefore, unpatentable. Applicants respectfully traverse the Examiner's rejection. A combination of these references in no way teaches or suggests that CLA was known or would have been expected to be a particularly effective treatment for diabetes.

Semenkovich is a reference which describes the relationship between diabetes and atherosclerosis, noting that in the vast majority of cases, individuals which exhibit symptoms of diabetes do not, in fact, develop premature vascular disease. See page 327 of Semenkovich, second column. In addition to the somewhat limited connection between diabetes and atherosclerosis is the fact that the mechanism for development of premature vascular disease is not particularly well understood. Indeed the title of the Semenkovich article is ***"The Mystery of Diabetes and Atherosclerosis*** *Time for a New Plot*." While Semenkovich teaches that antioxidants may be useful in addressing issues associated with oxidized lipoproteins in the development of atherogenesis, there is absolutely no disclosure that CLA *in particular* would be useful in the treatment of *diabetes*. Indeed, CLA is not even mentioned. Rather, the antioxidant of choice is ascorbate (page 332), a particularly potent antioxidant, which has significantly different physicochemical characteristics compared to CLA. Even a suggestion in Semenkovich that antioxidants (ascorbate) might be useful in reducing lipoprotein oxidation products and therefore, may play a beneficial role in limiting atherogenesis, does not evidence that CLA as an antioxidant could play such a role. See, for the example, the previously enclosed Abstract of Berliner and Heinecke, *Free Radic. Biol. Med.*, 1996, 20(5):707-727 ("Berliner"), cited in Semenkovich (note *64)*, which clearly indicates that the mechanism of oxidation of lipoprotein is

promoted by several different systems, including protein-bound metal ions, thiols, reactive oxygen intermediates, lipoxygenase, peroxynitrite and myeloperoxidase. While Semenkovich may suggest the generic use of antioxidants to treat macrovascular disease in those limited number of diabetic patients in which such a condition occurs, there is absolutely no suggestion in Semenkovich that CLA should be used to treat atherosclerosis or that antioxidants should be used to treat diabetes *per se*. *Noted here is the fact that even Semenkovich acknowledges that only a limited number of diabetic patients actually are at risk for macrovascular disease, most likely based upon some genetic predisposition.*

A limited disclosure, Semenkovich cannot possibly be read to suggest the use of CLA as a treatment modality for diabetes. It cannot even be fairly said that Semenkovich suggests CLA as a treatment modality for macrovascular disease, because it is not clear from the disclosure of Semenkovich (which cites Berliner) or from Berliner itself, that CLA would be a particularly effective antioxidant, given the lack of understanding of the oxidative process in producing such a condition and the fact that ascorbate, a particularly potent antioxidant, is disclosed. Semenkovich is clearly a deficient reference.

Steinhart does nothing to cure the deficiencies of Semenkovich, other than to suggest that CLA *may be* (obvious to try) useful to treat macrovascular disease, which occurs in a limited number of diabetic patients. See Semenkovich at page 327. Steinhart discloses generally, that CLA is a natural antioxidant, which has important uses in the limitation of carcinogenesis and in certain instances perhaps, atherogenesis. Steinhart further discloses, on page 4 of the article, that rabbits and hamsters were fed cholesterol-supplemented diets, animals which also received CLA had lower levels of total and LDL (i.e., "bad") cholesterol in their blood and developed less atherosclerosis in their aortas. Thus, Steinhart, at best, teaches that CLA *may be* (i.e., obvious to try) useful in limited instances in reducing the tendency of hypercholesterolemia to develop further into atherosclerosis. Combining Semenkovich with Steinhart at best, merely suggests that CLA may be useful to treat atherogenesis, in instances where hypercholesterolemia, and in particular, high LDL levels, are present. Thus, the disclosure of Steinhart provides suggests an obvious to try approach to the treatment of atherosclerosis. However, even after more than six years since the publication of Steinhart and Semenkovich, CLA still is not recognized as a viable treatment modality for atherslerosis in patients, in the presence or absence of diabetes. Indeed, the enclosed paper and abstract, Khosla and Fungwe, *Current Opinions in* Lipidology, 12(1), pp. 31-34 (February, 2001) indicate that even in 2001, it was not clear that CLA was actually useful for treating atherogenesis and the animal models and related research do not permit such a conclusion. Khosla, at page 33, second column (enclosed). Thus, it is not obvious that CLA is actually useful for the treatment of atherosclerosis (note that human patients were not treated in Steinhart only laboratory test animals), only that it *may* be useful. Yet the art still does not yet recognize CLA in the treatment of atherosclerosis. Moreover, there is absolutely no disclosure or suggestion in Steinhart that CLA is useful for the treatment of diabetes (i.e., lowering glucose), or even that the type of atherosclerosis associated with diabetes would be treated by CLA, given the mechanistic complexicity of such a disease. In short, Steinhart does not even obliquely suggest that CLA can be used to treat diabetes.

It is noted that a coincident side effect of improving glucose control and reducing

glycated cellular products *may be* that the oxidation of LDL is reduced. One could speculate that this might be of benefit to a person by reducing atherosclerosis/cardiovascular disease (ASCVD) risk. This is the teaching of Steinhart and this is the basis for the teaching upon which the Examiner relies. However, the speculated benefit is not recognized by the art as an actual treatment and the art fails to recognize CLA as a *bona fide* treatment of atherosclerosis or to reduce atherogenesis. See, Khosla, enclosed. Moreover, regardless of the fact that CLA is not recognized in the art as a treatment of atherosclerosis is that there is absolutely ***no*** (ie., ***zero***) evidence that reducing oxidation of LDL leads to improved glucose control in people with diabetes. Thus, the combined disclosures of Semenkovich and Steinhart do not make out a cogent rejection of the instant invention.

Turning to Cook, Cook does not obviate the deficiencies of a combined disclosure in Semenkovich and Steinhart in failing to suggest the present invention. Cook has been discussed supra, and that discussion is referenced here. Cook teaches that CLA may be used to limit humoral based allergic reactions or to decrease white blood counts. There is not even an oblique reference in Cook to diabetes. Cook is inapposite to this discussion. Indeed, Applicants are somewhat baffled that Cook was even used in making this rejection.

In the present case, the Examiner relies on a hindsight construction to make an obviousness rejection. There is absolutely no teaching or suggestion that one can glean from the combined teachings of Semenkovich, Steinhard and Cook that evidences that CLA is useful or should be used to treat diabetes. Again, the Examiner has used the inventor's own teachings in the present specification against the present application in order to assert that the present invention is unpatentable. Indeed, without the teachings of the present specification, the Examiner would not even be aware that CLA could be used to treat diabetes. That is the hallmark of hindsight reconstruction. As discussed in great detail hereinabove, the CAFC has opined that this type of hindsight construction is impermissible. Thus, the present invention is clearly patentable over the references cited by the Examiner.

Indeed, current law does not support the Examiner's rejection of the instant application. If the alleged obviousness of a claimed invention is based on a combination of references, there must be a rigorous showing of a clear and particular suggestion, teaching, or motivation to combine the references relied upon. *In Re Dembiczak,* 50 U.S.P.Q.2d 1614 (Fed. Cir. 1999). Such evidence may come from the references themselves, the knowledge of those skilled in the art, or from the nature of the problem to be solved. While this showing may come from the prior art, as filtered through the knowledge of one skilled in the art, *Brown and Williamson Tobacco Corp., Inc. v. Philip Morris Inc.,* 56 U.S.P.Q. 2d 1456 (Fed. Cir. 2000), it is still subject to the rigorous requirement that the combination not be motivated by impermissible hindsight. *In Re Dembiczak, supra.* Further, there must be a particular showing that one of ordinary skill in the art would have believed there was a reasonable likelihood of success that the suggested combination of references would work to yield the claimed invention. *Brown and Williamson Tobacco Corp, supra.* In the present case, Applicants respectfully submit that the Examiner's has not made out a cogent case for the rejection of the present application.

The Examiner has failed to provide a rigorous showing of a clear and particular suggestion, teaching, or motivation to combine Semenkovich, Steinhart and Cook, to yield the present method treating diabetes. To the contrary, without any suggestion or motivation in the art, the Examiner has taken the teachings of Semenkovich, Steinhart and Cook and applied them to a method (treating atherosclerosis) which is not related to the instantly claimed method of treating diabetes. It is improper as a matter of law to select, modify and combine references, in essence to cherry-pick the disclosures, in this manner in the absence of clear evidence supporting the selection, modification, and combination to support the obviousness rejection. *In Re Dembiczak, supra.*

There is also no basis for the Examiner to characterize the instant invention as reflecting the mere combination of the teachings of the cited references, without engaging in an

impermissible hindsight reconstruction of the present invention. There is no lower threshold in establishing obviousness for food or food supplement related inventions; a uniform standard precludes reliance on hindsight in evaluating the patentability of an invention irrespective of complexity. *Panduit Corp. v. Dennison Mfg. Co.*, 1 U.S.P.Q. 2d 1593 (Fed. Cir.), *cert. denied*, 481 U.S. 1052 (1987). Based upon the foregoing, the present invention is patentable.

For the above reasons, Applicant respectfully asserts that the claims set forth in the present amendment are now in compliance with 35 U.S.C. Applicants respectfully submit that the present application is now in condition for allowance and such action is earnestly solicited.

Applicant has neither cancelled nor added any claims. No fee is therefore due for the presentation of this amendment. A petition for a two month extension of time is enclosed as is a request for continued examination. A check for the appropriate fee is enclosed. Small entity status is claimed for the present application.

Please credit any overpayment or charge any additional fee due to Deposit Account No. 04-0838.

Respectfully submitted,

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Conjugated linoleic acid: effects on plasma lipids and cardiovascular function.

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Conjugated linoleic acid is a collective name for mixtures of several positional and geometric conjugated dienoic isomers of linoleic acid, which have been shown to impact favorably on several biological processes, particularly carcinogenesis. Recent studies have clearly established that the c9, t11 and t10, c12 isomers have distinct biological effects. The latter may be of particular importance in affecting blood lipids. Because conjugated linoleic acid has been suggested to be anti-atherogenic, this review is focused on its effects on cardiovascular function. Careful scrutiny of the literature suggests that at present it is premature to assign any beneficial role to conjugated linoleic acid in terms of its ability to impact either blood lipids or atherogenesis.

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Conjugated linoleic acid: effects on plasma lipids and cardiovascular function

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Conjugated linoleic acid is a collective name for mixtures of several positional and geometric conjugated dienoic isomers of linoleic acid, which have been shown to impact favorably on several biological processes, particularly carcinogenesis. Recent studies have clearly established that the *c*9, *t*11 and *t*10, *c*12 isomers have distinct biological effects. The latter may be of particular importance in affecting blood lipids. Because conjugated linoleic acid has been suggested to be anti-atherogenic, this review is focused on its effects on cardiovascular function. Careful scrutiny of the literature suggests that at present it is premature to assign any beneficial role to conjugated linoleic acid in terms of its ability to impact either blood lipids or atherogenesis. Curr Opin Lipidol 12:31–34.

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Abbreviation

CLA conjugated linoleic acid

0957-9672

Introduction

Although references to conjugated linoleic acid (CLA) can be traced back to the 1960s [1], current interest in the health benefits of CLA started in the late 1980s, after it was identified as the anti-carcinogenic component present in fried ground beef [2]. Since then, an extensive literature has documented the anticarcinogenic effects of CLA [3,4•,5,6•,7,8]. In addition, there is some evidence that CLA is also anti-atherosclerotic [9,10], has beneficial effects on immune function [11,12], is a growth factor in rats [13], and may play a key role in helping to regulate body fat [14]. Whereas any one of these effects would be considered advantageous, the possibility that CLA affects all of these is fascinating. On top of this, the fact that the richest natural sources of CLA, meat and dairy products, are consumed by people worldwide has tremendous implications for public health. This article will focus on the effects of CLA on plasma lipids and cardiovascular function.

CLA is a collective name for mixtures of several positional and geometric conjugated dienoic isomers of linoleic acid, with the *c*9, *t*11 isomer being particularly important from the standpoint of diet [15]. The *t*10, *c*12 isomer is present in relatively high concentrations in several commercial CLA preparations. *In vivo*, *c*9, *t*12 is formed during the microbial biohydrogenation of linoleic and linolenic acid by a specific enzyme found in ruminant animals [16]. The *c*9, *t*11 and *t*9, *c*11 isomers are preferentially incorporated into various tissue phospholipids [17], and also occur in relatively high concentrations in several uncooked meats [15]. Evidence is mounting that the *c*9, *t*11 and *t*10, *c*12 isomers have distinct biological effects [4•]. It can be anticipated that future research efforts will focus on what each of the various isomers does.

Serum levels of CLA increase in humans consuming foodstuffs rich in CLA [18], with a 19–27% increase reported in individuals consuming a cheddar cheese supplement that provided ~170 mg CLA a day [19]. CLA is known to occur in a wide variety of foods that include ruminant meats, milk, other dairy products, partly hydrogenated vegetable oils and to a much lesser extent certain vegetable oils [15,20,21]. The *c*9, *t*11-isomer accounts for up to 80–90% of the total CLA in meats and dairy products, whereas this isomer accounts for less than 50% of total CLA in seafoods and vegetable oils. Although diet is the major source of CLA in humans, there is little information about typical CLA

consumption patterns. It is only in recent years that the CLA content of foods has become available [15,20]. On the basis of this information, typical US consumption has been estimated (based on the amounts present in meat and dairy products) to be of the order of several hundred milligrams per day [15], whereas a figure of ~400 mg per day has been reported for Germany [22]. As yet there is no systematic database for the CLA content of foods.

Conjugated linoleic acid, plasma lipids and cardiovascular function

Although CLA is suggested to be anti-atherogenic in several review articles, there are as yet only three reports detailing its effects on atherosclerosis. Lee *et al.* [9] fed rabbits semi-synthetic atherogenic diets (14% fat and 1% cholesterol) with or without CLA (~0.5 g/day per rabbit) for 22 weeks. CLA feeding was associated with significant reductions in total cholesterol, LDL-cholesterol and plasma triacylglycerol concentrations. CLA did not affect HDL-cholesterol concentrations *per se*; accordingly the decrease in LDL-cholesterol resulted in a significant reduction in the LDL-cholesterol:HDL-cholesterol ratio. Autopsy data revealed a consistent, albeit non-significant, tendency towards less atherosclerosis in the aortas of the animals fed CLA. In a recent review article, Kritchevsky [6•] provided information (from one of his studies that is as yet unpublished) that feeding 1% CLA as part of a semipurified atherogenic diet (0.2% cholesterol) had a significant beneficial impact on both the progression and regression of atherosclerosis. The effects on atherosclerosis were observed even though plasma lipids were unaffected. Nicolosi *et al.* [10] fed hamsters atherogenic diets for 11 weeks, and investigated three different levels of CLA (0.06, 0.11 and 1.1%). All levels of CLA significantly reduced total cholesterol and VLDL plus LDL-cholesterol concentrations, whereas HDL-cholesterol was not affected. Neither levels of CLA resulted in statistically significant reductions in fatty streak areas (measured in the aortic arch), although combined data from the three CLA-fed groups revealed a significant 26% reduction in fatty streak area compared with control animals. In both the rabbit and hamster studies, a commercial preparation of CLA (present as the free fatty acid) was used, which contained a mixture of CLA isomers.

In contrast to the above studies, Munday *et al.* [23••] recently reported that CLA promoted fatty streak formation in C57BL/6 mice. Animals were fed diets containing 0.5% CLA, 0.25% CLA plus 0.25% linoleic acid or a control diet with 0.5% linoleic acid. All diets contained 1% cholesterol. Although CLA-fed animals had a less atherogenic lipoprotein profile (tendency for lower total cholesterol, higher HDL-cholesterol, resulting in a lower ratio of total to HDL-cholesterol), the total aortic fatty streak area was significantly higher, suggesting that CLA promoted atherogenesis independent of plasma lipids. It is possible that any beneficial effects of CLA in this model were swamped by the very high levels of dietary cholesterol relative to the body weight of the animals. Alternatively, in this particular model, a higher level of CLA might be needed to see a beneficial effect on atherogenesis. The three studies evaluating CLA and atherogenesis, carried out in three different animal models, are thus inconclusive in predicting how CLA will behave in man. As succinctly discussed recently [24], there is at present no evidence in support of the anti-atherogenic effect of CLA.

In addition to the above, several studies have reported on the effects of CLA on plasma lipids. Stangl *et al.* [25•] investigated the effects of CLA on lipoproteins in female pigs. Animals were fed an experimental diet containing 1% CLA (mixture of CLA isomers; approximately 35% *c*9, *t*11 plus *t*9, *c*11, 18% *t*10, *c*12, 5% *t*9, *t*11 and 2% *c*9, *c*11). The α-tocopherol content of both control and test diets was equalized by adding α-tocopherol to the test diet. CLA feeding did not affect total plasma triacylglycerol or total cholesterol concentrations, but significantly increased the LDL-cholesterol:HDL-cholesterol ratio by 26% ($P<0.04$), reflecting a non-significant increase in LDL-cholesterol. Sugano *et al.* [26] found no difference in serum total cholesterol or HDL-cholesterol in rats fed diets containing 1% CLA.

The above studies of CLA action on plasma lipids used a mixture of isomers and the free fatty acid. The first study utilizing individual isomers of CLA incorporated into triglycerides was recently reported [27••]. In that study, hamsters were fed purified diets (30% from fat, 0.01% cholesterol), in which total CLA represented approximately 5% by weight. Three diets were used, one contained a CLA mix (2.4% *c*9, *t*11 and 2.4% *t*10, *c*12), one was based on the *c*9, *t*11 isomer (4.5% by weight), whereas the other contained the *t*10, *c*12 isomer (4% by weight). The interesting finding was that the CLA mix and the *t*10, *c*12 isomer decreased LDL-cholesterol, decreased VLDL triglyceride and lowered HDL-cholesterol concentrations, whereas the *c*9, *t*11 isomer had no such effect. That study thus seemed to suggest that the *t*10, *c*12 isomer is the key isomer that affects plasma lipid levels.

Mechanism of action of conjugated linoleic acid

Collectively, the above studies do not provide any clear-cut evidence for the effects of CLA on blood lipids or atherogenesis. Although CLA inhibited atherogenesis in the rabbit [9] and hamster [10], it increased atherogenesis in the mouse model [23••]. As far as blood lipids are concerned, LDL-cholesterol was either decreased [9,10,27••], increased [25] or unaffected [23••]. These

disparate effects no doubt reflect the different animal models used, as well as the fact that the isomers utilized were generally not clearly identified. In addition, differences in feeding regimens (e.g. different levels of cholesterol employed) would certainly have been a factor. Regardless of this, the decrease in LDL-cholesterol observed [9,10,27••] is consistent with both decreased apolipoprotein B secretion, observed in Hep G2 cells [28], and decreased intracellular triacylglycerol, observed in mouse adipocytes [29], after treatment with the *t*10, *c*12 CLA isomer. With regard to atherogenesis, there are as yet no convincing data to answer this question emphatically. Originally, CLA was thought to protect LDL from oxidation because it was a more potent antioxidant than α-tocopherol [17]. The antioxidant hypothesis was disproved, because it was found that CLA could not effectively protect membranes from oxidative modification under conditions of metal ion-dependent oxidative stress [30]. Although CLA may not function as a true antioxidant, it has been found that auto-oxidation of CLA produced furan fatty acids, which may protect against oxidant-mediated toxicity [31].

As CLA is incorporated into cell membrane phospholipids [17], it may modify their fluidity and exert its effects by altering intracellular events via one or more signal transduction pathway(s) or eicosanoid synthesis. Because CLA is a polyunsaturated fatty acid, it may compete with linoleic acid in the pathway of eicosanoid synthesis, via the cyclooxygenase or lipoxygenase pathways. A recent study [32] suggested that various isomers of CLA can be precursors for eicosanoid synthesis as they are elongated and desaturated in a manner similar to linoleic acid. It thus seems feasible that these CLA-derived eicosanoids could affect a multitude of pathways involved in lipid metabolism. In this regard, prostaglandin E2 synthesis is known to be decreased by CLA [33,34]. The potential for CLA to affect intracellular lipid metabolism also comes from recent work on the stearoyl coenzyme A desaturase enzyme. The latter is responsible for desaturating palmitic and stearic acids to palmitoleic and oleic acid, respectively . It has been shown that hepatic stearoyl coenzyme A desaturase messenger RNA expression is decreased by CLA [35], and that this downregulation is specific for the *t*10, *c*12 isomer [36••]. In addition, the elegant studies by Moya-Camarena and colleagues [7,37] clearly established that CLA is both a ligand and an activator for the peroxisome proliferator-activated receptor α. This transcription factor modulates gene expression for several enzymes and proteins involved in lipid metabolism, including lipoprotein lipase [38], fatty acid binding protein [39], and acyl coenzyme A oxidase [40]. Furthermore, different CLA isomers have different potencies for peroxisome proliferator-activated receptor α [37].

Conclusion

Although there is a vast database on the anticarcinogenic properties of CLA, knowledge of its effects on lipids, atherogenesis and other aspects of cardiovascular function is still limited, and as exemplified by the data on atherogenesis and blood lipids, there is no clear consensus. However, almost every review article published seems to suggest that CLA inhibits atherogenesis. As elegantly pointed out by Rudel [24], the published data do not permit such a conclusion. After almost a decade of active research, there are no published data on humans or non-human primates. In no species has a consistent, reproducible, dose-dependent effect of CLA been established. Until such data are published, the debate on the involvement of CLA in cardiovascular function and blood lipids will continue. The availability of different CLA isomers should help to resolve some of the questions.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Bartlet JC, Chapman DG. Detection of hydrogenated fats in butter fat by measurements of *cis trans* conjugated unsaturation J Agric Food Chem 1961; 9:50–53.

2 Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 1987; 8:1881–1887.

3 Pariza MW, Park Y, Cook ME. Conjugated linoleic acid and the control of cancer and obesity. Toxicol Sci 1999; 52:107–110.

4 • Pariza MW, Park Y, Cook ME. Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc Soc Exp Biol Med 2000; 223:8–13.
The authors provide some interesting hypotheses on the mode of action of CLA.

5 MacDonald HB. Conjugated linoleic acid and disease prevention: a review of current knowledge [suppl]. J Am Coll Nutr 2000; 19:111S–118S.

6 • Kritchevsky D. Antimutagenic and some other effects of conjugated linoleic acid. Br J Nutr 2000; 83:459–465.
The author, in reviewing the data on atherogenesis, provides some interesting unpublished data from his laboratory.

7 Moya-Camarena SY, Belury, MA. Species differences in the metabolism and regulation of gene expression by conjugated linoleic acid. Nutr Rev 1999; 57:336–340.

8 Sebedio JL, Gnaedig S, Chardigny JM. Recent advances in conjugated linoleic acid research. Curr Opin Nutr Metab Care 1999; 2:499–506.

9 Lee KN, Kritchevsky D, Pariza, MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 1994; 108:19–25.

10 Nicolosi RJ, Rogers EJ, Kritchevsky D, *et al.* Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 1997; 22:266–277.

11 Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poultry Sci 1993; 72:1301–1305.

12 Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem Biophys Res Commun 1994; 198:1107–1112.

13 Chin SF, Storkson JM, Albright KJ, *et al.* Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. J Nutr 1994; 124:2344–2349.

14 Park Y, Albright KJ, Liu W, *et al.* Effect of conjugated linoleic acid on body composition in mice. Lipids 1997; 32:853–858.

15 Chin SF, Liu W, Storkson JM, *et al.* Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Comp Anal 1992; 5:185–197.

16 Kepler CR, Hirons KP, McNeill JJ, Tove SB. Intermediates and products of the biohydrogenation of linoleic acid by butyrivibrio fibrisolvens. J Biol Chem 1966; 241:1350–11354.

17 Ha YL, Storkson J, Pariza MW. Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res 1990; 50:1097–1101.

18 Britton M, Fong C, Wickens D, Yudkin J. Diet as a source of phospholipid esterified 9,11-octadecadienoic acid in humans. Clin Sci 1992; 83:97–101.

19 Huang YC, Luedecke LO, Shultz TD. Effect of cheddar cheese consumption on plasma conjugated linoleic acid concentrations in men. Nutr Res 1994; 14, 373–386.

20 Shantha NC, Ram LN, O'Leary J, *et al.* Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J Food Sci 1995; 60:695–697.

21 Parodi PW. Conjugated octadienoic acids of milk fat. J Dairy Sci 1977; 60:1550–1553.

22 Fritsche J, Steinhart H. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z Lebensm Unters Forsch A 1998; 206:77–82.

23 •• Munday JS, Thompson KG, James KAC. Dietary conjugated linoleic acids promote fatty streak formation in the C57BL/6 mouse atherosclerosis model. Br J Nutr 1999; 81:251–255.
This study shows that CLA actually promotes atherogenesis in this mouse model, despite producing a favorable plasma lipid profile. This is the first study to show that CLA negatively impacts atherogenesis.

24 Rudel LL. Atherosclerosis and conjugated linoleic acid. Br J Nutr 1999; 81:177–179.

25 • Stangl GI, Muller H, Kirchgessner M. Conjugated linoleic acid effects on circulating hormones, metabolites and lipoproteins, and its proportion in fasting serum and erythrocyte membranes of swine. Eur J Nutr 1999; 38:271–277.
Feeding a mixture of CLA isomers produces adverse plasma lipids in the pig. This is the first report to suggest that CLA may have adverse effects on plasma lipids, similar to those seen with trans monounsaturated fatty acids.

26 Sugano M, Tsujita A, Yamasoki M, *et al.* Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. J Nutr Biochem 1997; 8:38–43.

27 •• de Deckere EAM, van Amelsvoort JMM, McNeill GP, Jones P. Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster. Br J Nutr 1999; 82:309–317.
The only report to date to look at the effects of specific CLA isomers on plasma lipids. It is noted that the *t*10, *c*12 isomer favorably impacts plasma lipids, whereas the *c*9, *t*11 isomer had no such effect.

28 Yotsumoto H, Hara E, Naka S, *et al.* 10 *trans*, 12 *cis* – linoleic acid reduces apolipoprotein B secretion in Hep G2 cells. Food Res Int 1999; 31:403–409.

29 Park Y, Albright KJ, Storkson JM, *et al.* Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 1999; 34:235–241.

30 Van den Berg JJM, Cook NE, Tribble DL. Reinvestigation of the antioxidant properties of conjugated linoleic acid. Lipids 1995; 30:599–605.

31 Yurawecz MP, Hood JK, Mossoba MM, *et al.* Furan fatty acids determined as oxidation products of conjugated octadienoic acid. Lipids 1995; 30:595–598.

32 Sebedio JL, Juanenda P, Dobson G, *et al.* Metabolites of conjugated isomers of linoleic acid (CLA) in the rat. Biochim Biophys Acta 1997; 1345:5–10.

33 Li Y, Watkins BA. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostagalndin E2 biosynthesis in rats. Lipids 1998; 33:417–425.

34 Liu KL, Belury MA. Conjugated linoleic acid reduces arachidonic acid content and PGE2 synthesis in murine keratinocytes. Cancer Lett 1998; 127:15–22.

35 Lee KN, Pariza MW, Ntambi JM. Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. Biochem Biophys Res Commun 1998; 30:817–821.

36 •• Choi Y, Kim YC, Han YB, *et al.* The *trans*-10, *cis*-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. J Nutr 2000; 130:1920–1924.
In characterizing the effects of CLA on Scd-1 mRNA expression, this study is the first to show isomer-specific effects.

37 Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, *et al.* Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARα. J Lipid Res 1999; 40:1426–1433.

38 Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J Lipid Res 1996; 37:907–925.

39 Sterschele PF, Van den Heuvel JP, Davis JW, *et al.* Induction of hepatic acyl-CoA binding protein and liver fatty acid binding protein by perfluorodecanoic acid in rats. Lack of correlation with hepatic long chain acyl-CoA levels. Biochem Pharmacol 1994; 48:955–966.

40 Belury MA, Moya-Camareno SY, Liu KL, Vanden Heuvel JP. Dietary conjugated linoleic acid induces peroxisome-specific enzyme accumulation and ornithine decarboxylase activity in mouse liver. J Nutr Biochem 1997; 8:579–584.